Inactivation of *Listeria innocua* on Frankfurters That Contain Potassium Lactate and Sodium Diacetate by Flash Pasteurization

C.H. SOMMERS, D.J. GEVEKE, AND X. FAN

ABSTRACT: ***Listeria monocytogenes*, a psychrotrophic foodborne pathogen, is a recurring postprocess contaminant on ready-to-eat meat (RTE) products, including frankfurters. Potassium lactate (PL) and sodium diacetate (SDA) are FDA-approved antimicrobials that inhibit the growth of *L. monocytogenes* when incorporated into the formulation of fine emulsion sausage. Flash (steam) pasteurization (FP) has been shown to reduce levels of *L. monocytogenes*, and its surrogate *L. innocua*, on frankfurter surfaces. The ability of FP to inactivate and prevent the growth of the *L. monocytogenes* surrogate *L. innocua* in a pilot plant setting was investigated. FP treatment (1.5 s, 121 °C) of single layers of frankfurters that were surface-inoculated with either 5, 4, or 3 log CFU/g of *L. innocua* immediately before FP (1.5 s, 121 °C) resulted in log reductions of 1.97 (± 0.11), 2.03 (± 0.10), or 2.07 (± 0.14), respectively. Inoculum level had no effect on the inactivation of *L. innocua*. Following 8 wk of refrigerated storage (4 °C), *L. innocua* levels decreased by 0.5 log in non-FP-treated frankfurter packs, while the 2 log reduction of *L. innocua* was maintained for FP-treated frankfurters. FP (1.5 s, 121 °C) had no effect on frankfurter color or texture. Because the numbers of *L. monocytogenes* associated with contaminations of ready-to-eat meats are typically very low, the use of FP in combination with PL and SDA has the potential to reduce the number of frankfurter recalls and foodborne illness outbreaks.**

Keywords: flash pasteurization, frankfurters, *Listeria* potassium lactate, sodium diacetate

Introduction

Listeria monocytogenes is an occasional postprocess contaminant on ready-to-eat (RTE) meat products, including frankfurters; a number of foodborne illness outbreaks have been attributed to *L. monocytogenes* (Barnes and others 1989; Anonymous 1998, 2001; Mead and others 1999; FDA 2001), and the incidence of *Listeria* infections in 2005 was higher than its low point in 2002 (Anonymous 2006). *L. monocytogenes* is capable of growth at refrigerated temperatures and in high salt environments, which allows it to proliferate during long-term cold storage (Smith 1996). Because of the high mortality rate associated with infection, especially among at-risk populations, *L. monocytogenes* is given a zero tolerance in ready-to-eat meat products in the United States (USDA 1989; Gerba and others 1996). In the last 3 y (2003 to 2006) there have been 8 Class I recalls of frankfurters, not including other ready-to-eat (RTE) meats, due to contamination with *L. monocytogenes* (USDA FSIS 2006).

In a recent survey of RTE food products, *L. monocytogenes* was detected in 0.89% of deli meat products. However, 39% of the samples were contaminated with less than 0.1 CFU/g *L. monocytogenes*, 73% contained less than 1.0 CFU/g of the bacterium, and 87% contained less than 10 CFU/g of *L. monocytogenes* (Gombas and others 2003). The infectious dose for *L. monocytogenes* is currently unknown. While the health consequences of listeriosis are serious, it has been noted that low levels of *L. monocytogenes* in foods equal low risk (Chen and others 2003). Therefore, use of intervention technologies that produce even modest reductions in bacterial number, in combination with antimicrobial compounds, has the potential to reduce the number of frankfurter recalls and *L. monocytogenes*-associated foodborne illness outbreaks.

In order to prevent the contamination of frankfurters by *L. monocytogenes*, and to ameliorate the consequences due to consumption of accidentally contaminated frankfurters, the USDA Food Safety Inspection Service (FSIS) has recommended thorough plant sanitation and testing for the presence of *Listeria* spp. as part of an effective Hazard Analysis and Critical Control Point (HACCP) Plan. Postprocess interventions, either alone or in combination with antimicrobial compounds, may be used to inactivate and prevent the proliferation of *L. monocytogenes* on RTE meat products. Both potassium lactate (PL) and sodium diacetate (SDA) have been approved by the U.S. Food and Drug Administration (FDA) for use in RTE meat products (FDA 2000), and combinations of the two can effectively inhibit the growth of *L. monocytogenes* on RTE meats during long-term refrigerated storage (Mbandi and Shelef 2001; Seman and others 2002; Sommers and others 2003). Flash pasteurization (FP), a process that has recently been commercialized, uses short pulses of steam to decontaminate the surfaces of fine emulsion sausages such as frankfurters immediately before packaging (Murphy and others 2005a, 2005b, 2006).

Previous studies conducted in a pilot plant setting using FP have used *L. innocua* as a nonpathogenic surrogate microorganism in place of *L. monocytogenes* (Kozempel and others 2000; Sommers and others 2002; Murphy and others 2005a, 2006). The efficacy of FP when used in combination with the commonly used antimicrobials SDA and PL has not been reported. The purposes of this study were to (1) determine the effect of inoculum level on the ability of

MS 20070636 Submitted 8/16/2007, Accepted 9/14/2007. *Authors Sommers, Geveke, and Fan are with Eastern Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038, U.S.A. Direct inquiries to author Sommers (E-mail: christopher.sommers@ars.usda.gov).*

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Dept. of Agriculture.

doi: 10.1111/j.1750-3841.2007.00635.x

FP to inactivate the *L. monocytogenes* surrogate *L. innocua* on the surface of frankfurters that contain the antimicrobials PL and SDA in an open-air pilot plant setting; (2) determine the growth potential of *L. innocua* on FP-treated frankfurters that contained SDA and PL during refrigerated storage; and (3) determine the effect of FP on frankfurter color and texture.

Materials and Methods

Frankfurters

Freshly manufactured frankfurters were purchased from a local manufacturer. The frankfurters consisted of beef, pork, water, salt, flavoring, paprika, sodium phosphate, SDA (0.07%), PL (1.13%), sodium erythorbate, and sodium nitrate and were 25% fat. Frankfurters were stored at −20 °C and thawed overnight in a refrigerator for experimentation the following day.

L. innocua

Three *L. innocua* strains (51742, 33090, 33091) were obtained from the American Type Culture Collection (Manassas, Va., U.S.A.). The strains were propagated on tryptic soy agar (BD-Difco Laboratories, Sparks, Md., U.S.A.) at 37 °C and maintained at 0 to 2 °C until ready for use. Identity of *Listeria* was confirmed by Gram stain followed by analysis with Gram Positive Identification (GPI) cards using the Vitek Automicrobic System (bioMerieux Vitek Inc., Hazelwood, Mo., U.S.A.).

L. innocua propagation and inoculation

Each *L. innocua* strain was cultured independently in 30-mL tryptic soy broth (Difco) in baffled 50-mL sterile tubes at 37 °C (150 rpm) for 18 h. The cultures were then sedimented by centrifugation (1735 × *g* for 10 min) and resuspended as a mixture in 100 mL of Butterfield's Phosphate Buffer (BPB) (Applied Research Inst., Newtown, Conn., U.S.A.). Refrigerated gamma-irradiated frankfurters were then placed on a sterile surface, rolled in 1.0 mL of inoculum (either 10^5, 10^4, or 10^3 CFU/g), and allowed to dry in the refrigerator for approximately 30 min prior to FP.

Flash pasteurization

To assess the effect of inoculum level on the FP inactivation process, the surface-inoculated frankfurters were loaded into open preformed trays at the inlet of the flash pasterurization prototype unit (Alkar-RapidPak, Lodi, Wis., U.S.A.) as a single layer of 4 frankfurters. The frankfurters were then exposed to steam treatments (121 °C) for 1.5 or 3.0 s. The frankfurters were then placed in sterile nr 400 stomacher bags which were stored in an ice–water bath for approximately 30 min prior to enumeration of *L. innocua*.

Following FP the samples were assayed for colony forming units (CFUs) by standard pour plate procedures. Fifty milliliters of sterile BPB were added to a nr 400 stomacher bag that contained 4 frankfurters and shaken manually for 1 min (Sommers and Thayer 2000). The samples were then serially diluted in BPB, using tenfold dilutions, and 1 mL of diluted sample was pour plated using Palcam Medium (BD-Difco Inc.). Two 1-mL aliquots were plated per dilution. The Palcam plates were then incubated for 48 h at 37 °C prior to enumeration for CFU. Each experiment was conducted independently 3 times ($n = 3$).

Storage study

L. innocua was cultured as previously described, with the inoculum levels at 10^3 CFU/g being used for the storage study to simulate contaminations described by Gombas and others (2003). Following FP the frankfurter packs were then vacuum-packed (0.50 mmHg) in gas impermeable polynylon bags and refrigerated for 8 wk (4 °C). Three single layer packs were assayed for CFU/g every 2 wk for 8 wk using the methodology previously described.

Color analysis

Color analysis was then performed using a Hunter Lab Miniscan XE Meter (Hunter Laboratory Inc., Reston, Va., U.S.A.) (Sommers and others 2003). The meter was calibrated using white and black standard tiles. Illuminate D65, 10° standard observer, and a 2.5-cm port/viewing area were used. Results were from 3 independent experiments, with 12 readings taken per experiment.

Shear force

Cutting force of the frankfurters was measured using a Texture Technologies Corp. (Scarsdale, N.Y., U.S.A.) TA-XT2 Texture Analyzer. A TA-7 Warner-Bratzler blade was used with a test speed of 2.0 mm/s, 55-mm distance, and 20 g auto-trigger (Sommers and others 2003). Maximum shear force (*g*) results were from 3 independent experiments, with 12 readings taken per experiment.

Statistical analysis

Each experiment was conducted independently 3 times ($n = 3$). Descriptive statistics and analysis of variance (ANOVA) were performed using the descriptive statistics package of MS Excel (Microsoft Corp., Redmond, Wash., U.S.A.).

Results and Discussion

Relatively low numbers (CFU/g) of *L. monocytogenes* are typically associated with contaminations of frankfurters and other RTE meats, and low numbers of *L. monocytogenes* is equivalent to low risk (Chen and others 2003; Gombas and others 2003). Intervention technologies that can provide even modest inactivation of *L. monocytogenes*, when used in combination with antimicrobials, have the potential to significantly reduce the frequency of frankfurter recalls and foodborne illness outbreaks. PL and SDA are GRAS antimicrobials approved by the FDA for use in processed meats for inhibition of *L. monocytogenes* growth (FDA 2001). FP uses short pulses of steam to decontaminate the surfaces of fine emulsion sausages immediately prior to packaging and is currently used commercially for that purpose.

In this study, the ability of FP to inactivate the *L. monocytogenes* surrogate *L. innocua* on the surfaces of frankfurters that contained PL and SDA, arranged in single-layer packs, was investigated. This is the 1st report pertaining to the efficacy of the FP process when used in combination with the antimicrobials SDA and PL. *L. innocua* has been used as a surrogate microorganism in previous pilot-plant scale studies for FP decontamination of frankfurters (Kozempel and others 2000; Sommers and others 2002; Murphy and others 2005a, 2006). FP conditions (1.5 s, 121 °C steam) were used based on previous research and conditions of use in actual current commercial practice (Personal communication, Seth Pulsfus, Alkar-Rapid-Pak Inc.). FP treatment (1.5 s, 121 °C) of single layers of frankfurters that were surface-inoculated with either 5, 4, or 3 $\log_{10}$ CFU/g of *L. innocua* immediately before FP (1.5 s, 121 °C) resulted in log reductions of 1.97 (± 0.11), 2.03 (± 0.10), or 2.07 (± 0.14), respectively. Inoculum level had no effect on the inactivation of *L. innocua* as determined by ANOVA ($n = 3, \alpha = 0.05$). FP treatment time of 3.0 s (121 °C) resulted in 2.5 to 2.7 $\log_{10}$ reductions of *L. innocua* on the single layers of frankfurters. Increasing treatment time increased the inactivation of *L. innocua* as determined by ANOVA ($n = 3, \alpha = 0.05$).

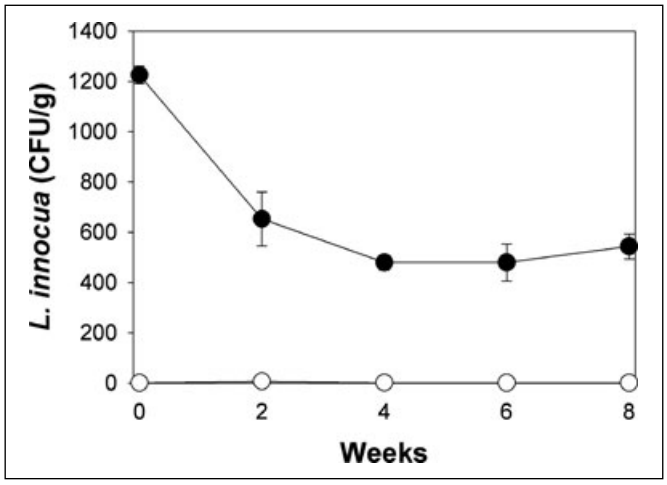

Figure 1—Proliferation of *Listeria innocua* during 8 wk refrigerated storage on frankfurters that contain potassium lactate and sodium diacetate with (open circles) and without (closed circles) flash pasteurization (1.5 s, 121 °C). Each experiment was conducted independently 3 times, with the standard error of the mean shown by error bars.

L. innocua levels during 8 wk storage at 4 °C decreased 0.5 $\log_{10}$ in non-FP-treated frankfurter packs while the 2 $\log_{10}$ reduction of *L. innocua* was maintained in FP-treated frankfurter packs (Figure 1). It should be noted that storage of frankfurters was in a common laboratory refrigerator that was repeatedly opened and closed during business hours during the 8-wk storage period, and that an 8-h power outage occurred during week 4 of the storage study. Conditions of mild temperature abuse may have occurred during the course of the storage study.

In order to evaluate the effect of FP on frankfurter quality, color and texture analyses were conducted. Hunter color *a*-values were 19.0 (± 1.57) and 20.3 (± 0.18), *b*-values 34.4 (± 1.84) and 35.3 (± 0.79), and *L*-values 59.6 (± 0.44) and 58.5 (± 0.43) for non-inoculated untreated and FP-treated frankfurters. Maximum shear force (*g*) was 1924 (± 60.8) and 1972 (± 41.0) for untreated and FP-treated frankfurters. There was no effect of FP on either frankfurter color or shear force as determined by ANOVA ($n = 3$, $\alpha = 0.05$).

Results obtained in this study are similar to those obtained in previous studies of flash pasteurization conducted under pilot-plant conditions. Murphy and others (2005a) obtained a 3 $\log_{10}$ reduction of *L. monocytogenes* and *L. innocua* in single-layer frankfurter prior to packaging using FP (1.5 s, 121 °C) using a commercial production Alkar-RapidPak FP/Intergrated Packaging Unit. Murphy and others (2006) obtained a 3 log reduction of *L. monocytogenes* and *L. innocua* on double-layer frankfurter packs treated with a commercial FP Alkar-RapidPak FP/Integrated Packaging Unit following an organic acid treatment, which inhibited growth of the pathogen for 14 and 19 wk at temperatures of 4 and 7 °C, respectively. Murphy and others (2005b) obtained both inactivation and growth suppression of *L. monocytogenes* during 47-d refrigerated storage using a combination of FP and liquid smoke.

Conclusions

Results obtained in these studies with either SDA/PL or organic acids and liquid smoke indicate that a 2 to 3 log reduction of *Listeria* spp. followed by growth inhibition of *Listeria* spp. for 2 to 3 mo could be obtained using FP under current commercial conditions to provide both inactivation and growth inhibition purposes. Frankfurter manufacturers should consider applying for FSIS Alternative 1 testing status for *Listeria* spp. if FP, when used in combination with appropriate antimicrobials, provides sufficient inactivation of and growth suppression of *Listeria* spp. during refrigerated storage.

Acknowledgments

We would like to thank Mr. O. Joseph Scullen and Ms. Kim Sokorai for technical assistance. We would like to thank Mr. Seth Pulsfus and Mr. Bryan Lemmenes of Alkar-RapidPak Inc. for their engineering and informational support assistance during the course of this study.

References

Anonymous. 1998. Multistate outbreak of listeriosis—United States, 1998. Morbid Mortal Wkly Rep 47:1085–6.
Anonymous. 2001. Listeria prevention and control. Food Proc 70:10.
Anonymous. 2006. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food -10 States, United States, 2005. 55(14):392–5.
Barnes R, Archer P, Strack J, Istre GR. 1989. Epidemiological notes and reports: listeriosis associated with consumption of turkey franks. Morbid Mortal Weekly Rep 38:268–9.
Chen Y, Ross WH, Scott VN, Gombas D. 2003. *Listeria monocytogenes*: low levels equals low risk. J Food Prot 66:570–7.
FDA. 2000. Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate and potassium lactate. Federal Register 65:3121–3.
FDA. 2001. Relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods; Draft risk assessment document and risk management action plan; availability. Federal Register 66:5515–6.
Gerba CP, Rose JB, Haas CN. 1996. Sensitive populations: who is at risk. Int J Food Microbiol 30:113–23.
Gombas DE, Chen Y, Clavero RS, Scott VN. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. J Food Prot 66:559–77.
Kozempel MF, Goldberg N, Scullen OJ, Radewonuk ER, Craig JC. 2000. Rapid hot dog surface pasteurization using cycles of vacuum and steam to kill *Listeria innocua*. J Food Prot 63:457–61.
Mbandi E, Shelef LA. 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella enteriditis* in meat by combinations of sodium lactate and diacetate. J Food Prot 64:640–4.
Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. 1999. Food-related illness and death in the United States. Emerg Infect Dis 5:607–25.
Murphy RY, Hanson RE, Feze N, Johnson NR, Scott LL, Duncan LK. 2005a. Eradicating *Listeria monocytogenes* from fully cooked franks by using an integrated pasteurization-packaging system. J Food Prot 68:508–11.
Murphy RY, Hanson RE, Johnson NR, Scott LL, Feze N, Chappa K. 2005b. Combining antimicrobials and steam treatment in a vacuum packaging system to control *Listeria monocytogenes* on ready-to-eat frankfurters. J Food Sci 70:M135–40.
Murphy RY, Hanson RE, Johnson NR, Chappa K, Berang ME. 2006. Combining organic acid treatment with steam pasteurization to eliminate *Listeria monocytogenes* on fully cooked frankfurters. J Food Prot 69:47–52.
Seman JL, Borger AC, Meyer JD, Hall PA, Milkowski AL. 2002. Modeling of the growth of *Listeria monocytogenes* in cured ready-to-eat meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. J Food Prot 65:651–8.
Smith LT. 1996. Role of osmolytes in adaptation of osmotically stressed and chill stressed *Listeria monocytogenes* grown in liquid media and on processed meat surfaces. Appl Environ Microbiol 62:3088–93.
Sommers CH, Thayer DW. 2000. Survival of surface-inoculated *Listeria monocytogenes* on commercially available frankfurters following gamma irradiation. J Food Safety 20:127–37.
Sommers CH, Fan X, Kozempel M, Radewonuk, R. 2002. Use of vacuum-steam-vacuum and ionizing radiation to eliminate *Listeria innocua* from ham. J Food Prot 65:1981–3.
Sommers CH, Fan X, Niemira BA and Sokorai K. 2003. Radiation (gamma) resistance and post-irradiation growth of *Listeria monocytogenes* suspended in beef bologna that contained sodium diacetate and potassium lactate. J Food Prot 66:2051–6.
USDA FSIS. 1989. Revised policy for controlling *Listeria monocytogenes*. Food Safety and Inspection Service, U.S. Dept. of Agriculture, Washington, D.C. Federal Register 54:22345–6.
USDA FSIS. 2006. FSIS recalls database. Available from: http://www.fsis.usda.gov/Fsis_Recalls/index.asp. Accessed Oct 1 2006.